

Genotyping of RAP^{-/-} x LDL Receptor ^{-/-} Mice by PCR

Purpose: To identify the mouse receptor associated protein (RAP) deficient gene from the wild type gene.

Gene Information: RAP^{-/-} mice were created by inserting a Neo cassette in Exon 1 (after the leader peptide) of the RAP gene. RAP gene is located on chromosome 5.

Primers:

1. RAP new > = 5'-TGATTGGTACCATCTCTGGGCTGG-3' (830-843 bp 5' untranslated region, upstream of exon 1)
2. RAP Neo < = 5'-GATTGGGAAGACAATAGAAGGCATGC-3' (3' Untranslated region of the Neo Cassete)
3. OIMR0690 (RAP>)= 5'-GCTAGTGCTGTTGTTGCTGC-3' (273-292 bp on Exon 1)
4. OIMR0691 (RAP<)= 5'- TTCTCTCGCGAGTACTTGCC-3' (325-344 bp on Exon 1)

(* > = forward primer and < = reverse primer)

PCR:

Reaction

1. Genomic DNA (2 µl)
2. Promega PCR Master Mix (2X; 10µl)
3. Primers (0.6µl)
4. Taq DNA polymerase (Promega, 0.2 µl)
5. PCR Water (3.6µl)

Total reaction volume is 20 µl.

Program

| | |
|------------------|--------------------------|
| 1. Cycle 1: | Step 1 - 94°C for 3 min |
| 2. Cycle 2 (12X) | Step 2 - 94°C for 20 sec |
| | Step 3 - 64°C for 30 sec |
| | Step 4 - 72°C for 35 sec |
| 3. Cycle 3 (25X) | Step 5 - 94°C for 20 sec |
| | Step 6 - 58°C for 30 sec |
| | Step 7 - 72°C for 35 sec |
| 4. Cycle 4: (1X) | Step 8 - 72°C for 2 min |
| 5. Cycle 5: (1X) | Step 9 - Hold at 4.0°C |

Expected bands by 2% TBE agarose gel electrophoresis:

| | |
|--------------------|------------------|
| RAP ^{+/+} | 78 bp |
| RAP ^{+/-} | 78 bp and 110 bp |
| RAP ^{-/-} | 110 bp |

Screening for double KO mice:

This strategy works well for double KO mice. Screen for the LDLR gene is done according to the protocol described by [\adlab\protocol\mouse\ldlrpcrjax.wpd](#)

Reference:

Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. Proc Natl Acad Sci U S A. 1995 May 9;92(10):4537-41.

File name: Z:\AAA PPG\Rateri Core\PCR protocols\RAP x LDLR Screening.wpd

Date of last update: 11/20/06 Person updating: DR



